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Dopamine–Acetylcholine Interaction in the Rat Lateral Hypothalamus in the Control of Locomotion

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PUIG DE PARADA, M., M. A. PARADA, P. RADA, L. HERNANDEZ AND B. G. HOEBEL. *Dopamine–acetylcholine interaction in the rat lateral hypothalamus in the control of locomotion.* PHARMACOL BIOCHEM BEHAV **66**(2) 227–234, 2000.—Pharmacological, neurochemical, and behavioral techniques were used to characterize DA–ACh interaction within the lateral hypothalamus (LH) in the context of locomotion, feeding behavior, and reinforcement. In Experiment 1, the muscarinic agonist carbachol injected in the LH increased locomotor activity in proportion to dose. In Experiment 2, the same doses of carbachol proportionately increased exctracellular DA in the nucleus accumbens (Nac) as monitored by brain microdialysis. Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) also increased. In Experiment 3, LH infusion by reverse microdialysis of the D_2 receptor blocker sulpiride released ACh in the LH in a dose–response manner. This suggested that sulpiride disinhibits ACh release via D_2 receptors in the LH and thereby facilitates behavior. Confirming this in Experiment 4, local LH atropine 5 min before sulpiride suppressed the locomotor response to sulpiride for about 20 min. These results suggest that sulpiride acts in the LH by disinhibiting a hypothalamic locomotor mechanism that is cholinergically driven and connected with the mesoaccumbens dopamine pathway. Given prior results that local sulpiride in the LH can induce hyperphagia and reward, this system may be involved in searching for food and rewarding feeding behavior. In conclusion, DA acts in the LH via D_2 receptors to inhibit cholinergic neurons or terminals that are part of an approach system for eating. © 2000 Elsevier Science Inc.

Microdialysis Neuroleptic drugs Nucleus accumbens Sulpiride Carbachol Atropine Microinjections

THE lateral hypothalamus (LH) is a dopaminergic terminal field (21,26,31,34). It has been shown that LH dopaminergic terminals belong to projections from dopamine (DA) cells within the hypothalamus or midbrain (10,24,27), and that DA exerts its functions mainly through D_2 receptors (32,33,38,40). The existence of cholinergic cell bodies and terminals has also been substantiated (43) in this area usually associated with thermoregulation (38), reward (3,30,37), locomotion (3,33,35), and consummatory behaviors (3,14,26,27,32,44).

Evidence shows that DA and acetylcholine (ACh) affect some LH functions in opposite ways, which suggests, in turn, an interaction between both neurotransmitters in that region. For example, local administration of DA agonists or cholinergic antagonists can reduce food and water intake (26,27,47), whereas cholinergic agonists or DA antagonists promote feeding and/or drinking (14,32,47). Microdialysis suggests that DA increases and ACh decreases in the LH during drinking (41), and indicates that these neurotransmitters covary in opposition during the development of water satiety. Additionally, these neurotransmitters display opposite effects on electrical unit activity associated with rewarding or aversive stimuli (12). Despite those facts, the nature of the DA–ACh interaction in the LH is largely unknown, and two possibilities should be explored. On the one hand, both neurotransmitters could exert independent actions on output neurons in the LH, and on the other, DA could influence cholinergic cells connected to output neurons. The present study explored this last possible interaction, which has already been demonstrated in other DA fields, including the striatum (STR) (4,8,9,19,28), nucleus accumbens (NAc) (45,49), and prefrontal cortex (PFC) (6,7).

Sulpiride, a dopaminergic D_2 receptor blocker (22) injected into the LH induces feeding and drinking (32), hyperthermia (38), locomotion (33,35), behavior reinforcement,

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and activation of the mesoaccumbens DA system (37). Because a large dose of the cholinergic agonist carbachol injected in the LH had a similar locomotion effect as sulpiride (35), both drugs were used in the present research to determine if the D_2 receptor system and the cholinergic system interact. The first experiment was intended to confirm the carbachol effect on locomotion and explore its pharmacological relevance through a dose–response analysis. In other experiments, microdialysis was used to detect a possible DA release in the NAc induced by carbachol injected into the LH, and the effect of sulpiride administered in the LH by reverse microdialysis on ACh release in that same area. To explore a behavioral consequence of the DA/ACh interaction, the last experiment tested muscarinic blockade of sulpiride-induced locomotion. In brief, it appears that within the lateral hypothalamus, DA D_2 receptors inhibit a cholinergic system that promotes behavior.

METHOD

Animals and Surgery

Seventy-four male Sprague–Dawley rats weighing between 380 and 420 g at the time of the surgery were individually housed at 21–23°C on a 15-h light, 9-h dark cycle (lights on at 700 h), with Purina chow pellets and tap water ad lib. Under ketamine (80 mg/kg) and pentobarbital (12 mg/kg) anesthesia rats were implanted with chronic guide shafts for one of the following procedures: (a) bilateral microinjections in the LH (27 gauge × 18 mm; $n = 35$); (b) bilateral microinjections in the LH (27 gauge × 18mm) combined with microdialysis in the right NAc (21 gauge × 10 mm; $n = 19$); or (c) microdialysis in the right LH (21 gauge × 10 mm; $n = 20$). The stereotaxic coordinates with the incisor bar placed 3.5 mm below the interaural line were: microinjections in the LH: 6.2 mm anterior to the interaural line, 1.6 mm lateral to the midsagittal sinus, and 6.6 mm perpendicularly below the surface of the skull (A 6.2, L 1.6, V 6.6); microdialysis in the right NAc: (A 10.2, L 1.2, V 3.5); microdialysis in the right LH: (A 6.2, L 1.6, V 3.6). No experiment was carried out before at least 1 week of postsurgical recovery.

Microdialysis

Microdialysis probes (17) were made of fused silica capillary (Polymicro Technologies Inc.) inside of 26-gauge stainless steel tubing ending in a 200-μm tip of cellulose dialysis fiber with a 6000 molecular weight cutoff and 3 mm long for the NAc or 2mm long for the LH. The tip of each probe protruded 5 mm from the lower end of the guide shaft. Probes were perfused with a Ringer's solution (146 mM NaCl, 4 mM KCl, and 1.2 mM CaCl2) at a flow rate of 1 μl/min, with the capillary tubing as the outlet and using PE10 for connections. Neostigmine (0.3 μM) was added to the Ringer's solution to improve ACh detection in perfusates from the LH.

For dopamine assays, dialysate samples from the NAc were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-EC). The 20-μl samples were injected into a 20-μl valve loop leading to a 10 cm long, 3.2 mm bore, 3 m ODS phase II column (Brownlee, Co.). The mobile phase contained 60 mM sodium phosphate monobasic, 100 μM EDTA, 1 mM heptanesulfonic acid, and 6% v/v methanol with pH adjusted to 3.6. The mobile phase flow rate was set at 1 ml/min provided by pressure generated by a single-piston pump (Model 220 B, SSI Inc., State College, PA). Neurochemical detection was performed by a Coulochem 5100 A (ESA Inc. Chelmsford, MA) with the guard cell set at +500 mV, conditioning electrode at +100 mV, and the second electrode at −400 mV. The order of elution was DOPAC, DA and HVA with retention times of 3.5, 5.2, and 10.5 min, respectively. Neurochemicals in each sample were measured by the ratio of unknown to standard peak heights, and the results expressed as pg/20 μl.

ACh from the LH was measured by reverse phase, HPLC-EC using a single piston pump and a pulse dampener (SSI Co.), a 20-μl sample loop and an amperometric detector (EG&G Princeton Applied Research Corp.). The mobile phase contained 200 mM potassium phosphate at pH 8.0. ACh was separated on an 8 cm C18 analytical column (Chrompack Inc., Raritan, NJ) and then converted sequentially to betaine and hydrogen peroxyde by an immobilized enzyme reactor (Chrompack Inc., with acetylcholinesterase and choline oxydase from Sigma Chemical Co., St. Louis, MO). The resultant hydrogen peroxide was oxidized on a platinum electrode (BAS Inc., Lafayette, IN) set at 0.5 V with respect to an Ag-AgCl reference electrode (EG&G Princeton Applied Res. Corp., Princeton, NJ). ACh in each sample was expressed as pmol/20 μl. The detection limit for ACh with this system was 20 fmol/20 μl standard sample. The retention time for ACh was between 7 and 8 mins.

Drugs

The following drugs obtained from Sigma Chemical Co. were used for the present experiments: Carbamylcholine chloride (carbachol), atropine sulfate and (±)sulpiride. Carbachol and atropine were dissolved in Ringer solution for intracerebral administration. Sulpiride for microinjections was prepared dissolving 27 mg of the racemic compound in 1.0 ml of a vehicle solution containing 0.9 ml of the perfusion Ringer and 0.1 ml of 1 N acetic acid. Vehicle control injection contained 0.1 ml of acetic acid per ml. The 12 mM sulpiride solution was prepared by dissolving 4.1 mg in 1.0 ml of a Ringer containing just 25 μl of 1 N acetic acid per ml. The lower concentrations were obtained by succesive dilutions of the 12-mM solution.

Experiment 1. Microinjections of Carbachol in the LH and Measurement of Locomotor Activity

Animals with guide shafts in the LH were given microinjections of carbachol [0 (vehicle), 0.5, 1, 2, or 4 μg/0.3 μl; $n = 5$ for each dose]. Microinjections were performed following the remote insertion developed (36) to avoid the perturbations that usually occur when handling the animals during the classical procedure technique. At the beginning of the experimental session the animal was attached to a flexible tether joined to a swivel, and a PE20 plastic guide (20 cm) was connected to each of the bilateral guide shafts in the LH. Locomotion was measured in a rectangular (20 × 35 cm) cage equipped with two infrared emitter-detector pairs placed 10 cm from the anterior and posterior edges of the lateral walls and 3 cm above the floor. Those photodetectors were interfaced to a microcomputer, and locomotor activity was measured as photobeam interruptions collected in 5-min sampling intervals. Habituation was obtained by placing the rat repeatedly in the cage for 1 h for at least 5 days before the experiment. The day of the experiment microinjections were performed after 1 h of additional habituation. The procedure was accomplished by sliding long silica glass injectors through the 20 cm plastic guides and down the implanted metallic guide shafts to the LH. Injectors were made of glass capillary tubing

(145 μm o.d.) that protruded exactly 2 mm beyond the tip of the intracerebral guide shaft to reach the intended target in the hypothalamus. The injection volume (0.3 μl) was delivered by a syringe pump for 1 min. Injectors, tether, and plastic guides were removed 30 s later, and the animals were allowed to move freely within the cage while the locomotor response was recorded.

Experiment 2. Microdialysis for DA in the NAc Combined With Microinjections of Carbachol in the LH

This experiment measured the effects of bilateral LH microinjections of carbachol [0 (vehicle, $n = 6$), 1 ($n = 6$) or 2 ($n = 7$) g/0.3 μl) on DA turnover in the NAc. At least 16 h prior to the collection of dialysates a dialysis probe was inserted into the right NAc, and two PE20 plastic tubes were connected to the LH metal guide shafts to serve as guides for the remote injector insertion the next day. The animal was then placed in a dialysis cage for the night with Ringer flowing at 0.1 μl/min. The next day food and water were removed just prior to the collection of dialysates. Samples were taken every 20 min for immediate analysis of DA, dihydroxyphenyl acetic acid (DOPAC), and homovanilic acid (HVA). After a stable baseline of at least three consecutive DA samples the animal received bilateral microinjections of carbachol in the hypothalamus, followed by four additional samples to assess the effects on the mesoaccumbens DA system.

Experiment 3. Microdialysis for ACh in the LH and Local Sulpiride Infusion

A sulpiride solution [0 (vehicle), 1.5, 3, 6 or 12 mM; $n = 4$ for each concentration] was infused for 20 min through a microdialysis probe inserted in the LH (reverse microdialysis), and the effects of these solutions on extracellular ACh were measured. For this procedure the line (PE10) connecting the swivel with the probe had previously been cut 33 mm from the probe inlet and the connection reinstated with a small piece of 30-gauge ss tubing. During microdialysis, after at least 3 baseline samples within 10% of each other, the main line was disconnected at this point and a PE10 segment (33 cm) containing 20 μl of the appropriate sulpiride solution was intercalated in the line. Microdialysis continued and four additional samples were taken for ACh measurement.

Experiment 4. LH Atropine Before Sulpiride

Atropine (5 μg/0.3 μl) was injected in the LH by the remote technique (see Experiment 1) 5 min before sulpiride (8 μg/0.3 μl). Locomotion was measured in the photocell cage as in Experiment 1.

Statistical Analysis

The relationship between the doses of carbachol and the magnitudes of the locomotor response were tested with linear regression analysis applied on behavioral data obtained during the first 30-min postinjection. Microdialysis data from each subject in each experimental session were normalized by converting peak heights to a percent of the mean of three consecutive baseline samples to overcome variability between subjects and allow appropriate comparisons. The effects were assessed in each group, and for each area, comparing the levels from the pretreatment samples with the mean levels of the samples obtained after LH drug administration by using one-way analysis of variance (ANOVA) for repeated measures followed by mean's regression coefficient comparisons to detect the significantly different points. Dose–response effects were tested by linear regression analysis. The same analysis were used to assess the dose–response effect for sulpiride infused through the microdialysis probe on ACh release in the LH. The interaction between LH atropine and sulpiride microinjections in regard to locomotion was explored by comparing the behavioral data after sulpiride with the behavioral data after atropine + sulpiride using one-way ANOVA.

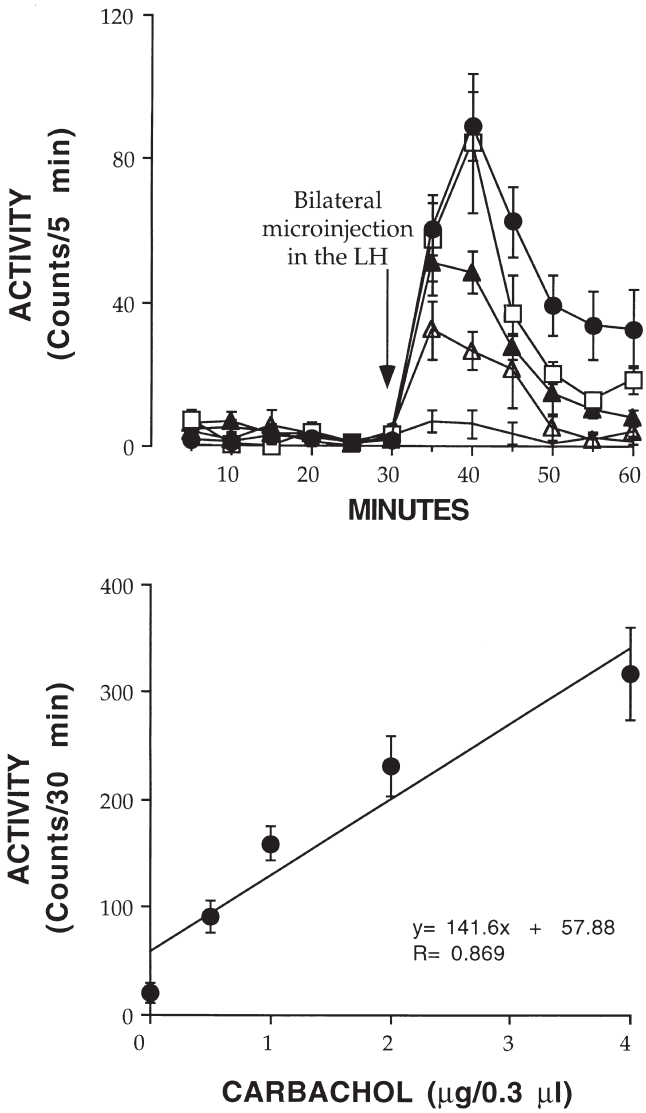

FIG. 1. Bilateral microinjections of different doses of carbachol [vehicle (plain line), 0.5 (open triangles), 1 (solid triangles), 2 (open squares), and 4 (solid circles) μg/0.3 μl; $n = 5$ in each group] into the lateral hypothalamus increased locomotion in rats. (Top) Temporal course of the phenomenon. The plotted data represent the cumulative number of interruptions (counts), in 5-min sampling intervals, of two photobeams strategically placed in the experimental cage. The arrow marks the injection time. (Bottom) Relationship between the carbachol dose and the cumulative activity displayed during the 30 min that followed the injection. The correlation was significant at $p < 0.0001$, $F(1724) = 70.94$, $r = 0.87$.

RESULTS

Experiment 1. Microinjections of Carbachol Into the LH Increase Locomotion

This experiment demonstrates that carbachol injected in the LH exhibits the same effect on locomotion as sulpiride. As shown in Fig. 1 (top graph), locomotion increased during the first three sampling intervals that followed each dose of carbachol. Regression analysis showed a significant correlation between the carbachol dose and cumulative activity during the 30-min postinjection period (bottom graph), $F(1, 24)$: 70.94, $R = 0.869$, $p < 0.0001$. Having found that LH carbachol is like sulpiride in causing locomotion, the next question was whether LH carbachol would release DA in the NAc as sulpiride does.

Experiment 2. Microinjections of Carbachol Into the LH Increase DA turnover in the NAc

Intrahypothalamic administration of carbachol (1 and 2 μg/0.3 μl) increased extracellular DA levels in the NAc in comparison to the DA levels after the vehicle injection (Fig. 2 top graph). The maximal increase was evident during the second sample postinjection. Therefore, data from these samples were used to run the regression analysis with graphic representation in Fig. 2 (bottom). The correlation was positive and statistically significant, $F(1, 18) = 9.33$, $r = 0.60$, $p < 0.01$. DOPAC (Fig. 3 top) and HVA (Fig. 3 bottom) also increased

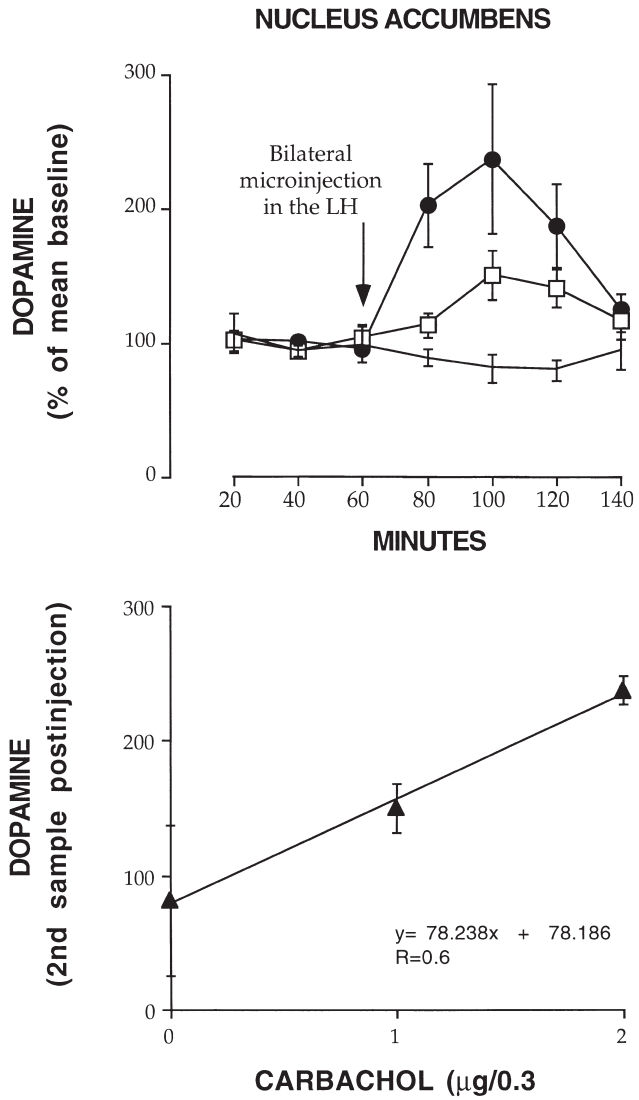

FIG. 2. Modifications of the extracellular DA levels in the NAC induced by bilateral intrahypothalamic microinjections of carbachol [1 (open square; $n = 6$) and 2 (solid circles; $n = 7$) μg/0.3 μl] or its vehicle (plain line; $n = 6$). (Bottom) Relationship between the carbachol doses and the DA levels from the second postinjection samples. The correlation was significant at $p < 0.01$, $F(1518) = 9.33$, $r = 0.60$.

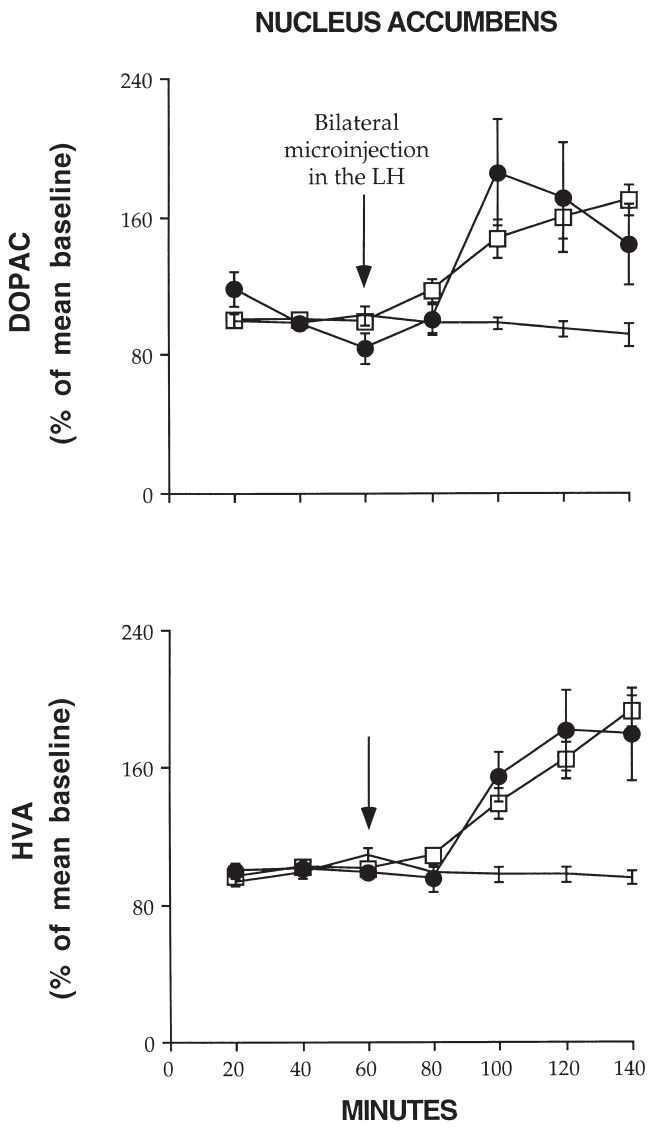

FIG. 3. Modifications of the extracellular levels of DOPAC (top) and HVA (bottom) in the NAC as a consequence of the bilateral intrahypothalamic administration of carbachol [1 (open squares; $n = 6$) and 2 (solid circles; $n = 7$) μg/0.3 μl] or its vehicle (plain line; $n = 6$). The increases in the extracellular levels of both metabolites and after both doses were statistically significant starting from the second postinjection sample. No correlation was found between the carbachol dose and the magnitude of the increase for either metabolite.

in the NAc as a consequence of the carbachol injections in the LH, although the increases for these metabolites in the NAc were not correlated with the doses of the drug. This finding that carbachol in the LH activates the mesoaccumbens DA system, like sulpiride, suggested for the next experiment that LH sulpiride might release ACh in the LH.

Experiment 3. Sulpiride Infusion Into the LH Increases Local ACh Release

The top graph in Fig. 4 shows that sulpiride infusion by reverse microdialysis increased ACh release in proportion to drug concentration in the perfusion fluid. This effect was maximal 40 min after the beginning of the infusion, and persisted for at least 40 more min. The regression analysis performed on the data from the second postinfusion sample revealed a good positive correlation with the sulpiride concentration in the perfusion fluid (Fig. 4, bottom graph), $F(1, 18) = 12.5$, $r = 0.64$, $p < 0.005$. Given this finding that LH sulpiride increases local extracellular ACh, we hypothesized that a cholinergic antagonist might block sulpiride's behavioral effect.

Experiment 4: Atropine Attenuates Locomotion Induced by Sulpiride When Both Drugs Are Administered Into the LH

This experiment corroborates previous findings showing that intrahypothalamic injections of sulpiride promote locomotion (33,35). As shown in Fig. 5, a bilateral microinjection of this D_2 blocker (8 μg) increased locomotion almost immediately when preceded by a control Ringer injection; however, the increase in locomotion was inhibited for at least 15 min when atropine (5 μg) was previously administered. The lack of effect of this last drug on spontaneous locomotion when administered alone had been previously reported (35), it was therefore considered unnecessary to replicate that previous finding in the context of the present study. One-way ANOVA showed that the difference in locomotion comparing the two treatment combinations was statistically different at 5 min [29.2 + 7 vs. 1.6 + 1.6 counts/5 min; $F(1, 8) = 14.4$, $p < 0.005$]; 10 min [31.2 + 2.8 vs. 3.8 + 2.4 counts/5min; $F(1, 8) = 52.74$, $p < 0.0001$], and 15 min [32 + 5 vs. 10.6 + 2.9 counts/5 min; $F(1, 8) = 13.5$, $p < 0.01$] after the sulpiride injection. This difference was also evident using the cumulative data for the first 20 min postsulpiride [118 + 13 vs. 35.4 + 5.5 counts/20 min; $F(1, 8) = 34$, $p < 0.0005$]. Atropine attenuation of the hyperlocomotion was almost total during the first 10 min after the sulpiride injection; however, the hyperactivity started 5 min later and increased progressively reaching the maximal levels at the end of the studied period (30 min postinjection).

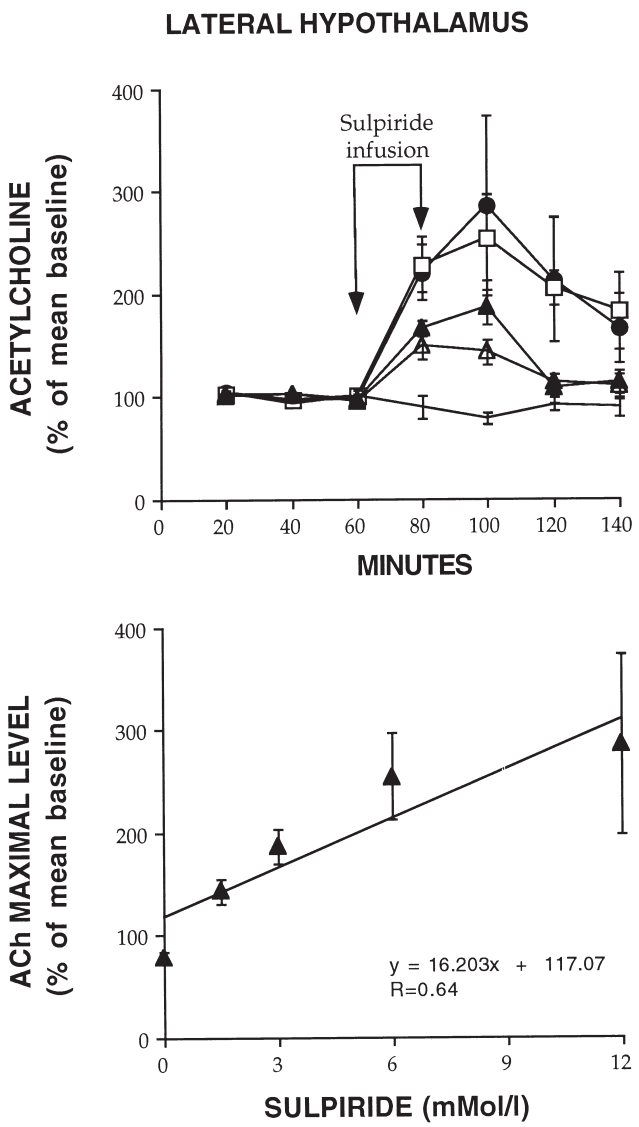

FIG. 4. Top.- Temporal course of the modifications in the extracellular ACh levels induced in the rat LH by the 20-min infusion of several sulpiride solutions [1.5 (open triangles), 3 (solid triangles), 6 (open squares), and 12 (solid circles) mM; $n = 4$ in each group] or plain Ringer (plain line). (Bottom) Relationship between the sulpiride concentration in the solution and the maximal ACh levels attained after the infusion (first sample postinfusion). The correlation was statistically significant at $p < 0.005$, $F(1,8) = 12.5$, $r = 0.64$.

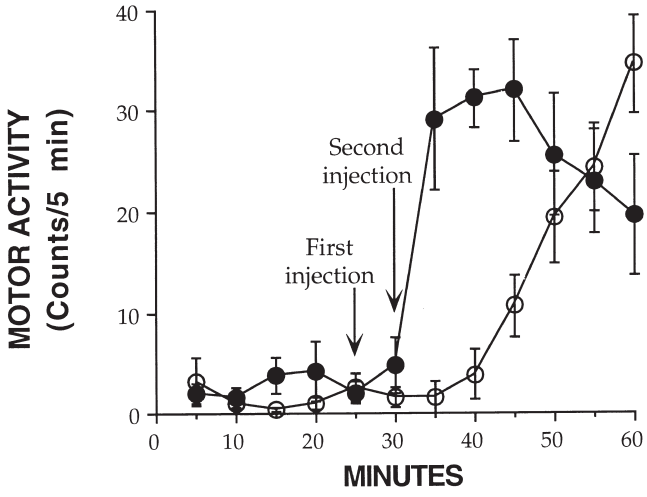

FIG. 5. Temporal course of the locomotor response induced by bilateral microinjections of sulpiride (8 μg/0.3 μl) into the rat LH. A robust locomotor response started immediately if sulpiride had been preceded 5 min before (first injection) by a local Ringer injection (solid circles; $n = 5$). When atropine (5 μg) was present in the first injection the sulpiride effect was totally suppressed during the first 15 min, although it recovered later (open circles; $n = 5$).

Thus, atropine pretreatment significantly delayed the onset of sulpiride-induced hyperactivity.

DISCUSSION

Some neurons within the LH are apparently involved in locomotion. Their existence can be inferred from three different lines of evidence. Electrical (3) and chemical (32,33,35) stimulation of this area induce locomotion and exploration, and its destruction with bilateral electrolytic lesions causes akinesia (29). However, the exact role and the functional significance of this LH locomotor mechanism have not been established, in part because the effects of lesions are largely attributed to destruction of DA fibers of passage and the resultant parkinsonian symptoms. It would seem, nonetheless, that its neurons belong to a circuit that becomes active under special circumstances, as previously suggested (35), because kainic acid destruction of LH cell bodies does not impair spontaneous locomotion (15). The locomotor response induced by sulpiride injected in the LH is a component of a more general state of psychomotor activation that includes rearing and sniffing, and that resembles the psychomotor agitation induced by psychostimulant drugs (32,33,35). The increase in DA turnover in the mesoaccumbens pathway after LH sulpiride injections, as well as the self-administration of this drug in the LH (37) suggest that both the sulpiride-induced locomotion and the locomotion triggered by most drugs of abuse (13,51) represent behavioral manifestations of the neurophysiological processes subserving behavior reinforcement. A related interpretation has been put forward based on the fact that DA injected in the LH reduces locomotion and exploration in food-and water-deprived animals, but not in animals placed for the first time in a novel environment. It was suggested, therefore, that LH mechanisms are involved in triggering locomotion related to the procurement of food and water (35). The locomotion triggered by carbachol in Experiment 1 confirms a previous observation and suggests the existence of cholinergic receptors in the LH normally involved in motor functions (35). The reliability of this pharmacological effect can be inferred from the significant dose–response relationship starting from a relatively low dose of the cholinergic agonist. The cholinergic locomotor response has the same characteristics and is almost undistinguishable from that induced by D_2 antagonist sulpiride. The mesoaccumbens DA system has been thought of as a common pathway subserving locomotion triggered by different means (20,23,25,48,51). This report shows that carbachol increases extracellular DA in the NAc and shares with sulpiride the activation of the dopaminergic mesolimbic projections. This suggests that both drugs probably use the same mechanism to induce locomotion.

Such a mechanism for locomotion in the LH implies the existence of direct or indirect connections between the LH and the VTA. Indirect connections via the lateral habenula, the locus coeruleus and some mesencephalic cholinergic cell groups have been proposed (16,42). More direct connections have been anatomically demonstrated in studies tracing axonal degeneration to the VTA after electrolytic lesions in the LH (18,52,), anterograde transport of tritiated aminoacids from the LH to the VTA (46), and retrograde transport of horseradish peroxidase from the VTA to the LH (39). Thus, it is conceivable that output neurons establishing LH–VTA connections bear muscarinic receptors, whose activation by carbachol yields an increase in the firing rate of this pathway, which in turn activates DA cells in the VTA.

These DA neurons in the VTA have D_2 autoreceptors and receive cholinergic activating projections from midbrain cell groups Ch. 5 and 6 (11,53–55), so it could be argued that sulpiride or carbachol injected in the LH acts by diffusion to the VTA. However, this possibility is very unlikely on the grounds that first, the distance for diffusion is too long to account for the locomotion that starts almost immediately after sulpiride or carbachol injections in the LH; second, sulpiride induces DA release in the NAc when injected in the LH, but not when injected in the posterior hypothalamus near to the VTA (37); and third, sulpiride in the present experiment was applied without pressure by using reverse dialysis to eliminate pressure-induced spread.

Interactions between DA and ACh have been demonstrated in several brain areas. Such interactions have been widely substantiated in the STR (4,8,9,19) in the NAc (45,49) and less frequently in the PFC (6,7). D_2 receptors have been positively identified on ACh neurons in the STR (5,28), and a great deal of pharmacological evidence indicates that they mediate the inhibitory action that DA exerts on cholinergic neurons in the NAc (45,49) and STR (4,8,9). D_2 receptors have also been identified in the LH (40). They might be involved in tonic inhibition of ACh release, because local sulpiride infusion increased extracellular levels of ACh in this report. ACh release could be one of the neurochemical consequences responsible for the spectrum of responses induced by sulpiride when it is injected in the LH. In the present report, a cholinergic drug mimicked sulpiride in regard to locomotion and DA release in the NAc. In addition, the cholinergic blocker atropine attenuated the locomotor response induced by sulpiride. It appears, therefore, that D_2 blockade disinhibits ACh release, which causes locomotion unless the ACh receptors are blocked with atropine.

Suppression of sulpiride-induced locomotion by atropine lasted just about 15 min, after which the animals became hyperactive. This time course might be due to the relative potency of atropine vs. sulpiride or the relative degradation rates, but the most plausible explanation for the late emergence of locomotion after the combined treatment probably relies on the different diffusion rate of both compounds within the brain combined with a high rate of dissociation of atropine from its receptors. A method was recently introduced to estimate the migration rate of substances in the brain tissue, through the analysis of the migration rate of their neurochemical effects using two microdialysis probes–one for drug infusion and neurochemical sampling, and the other just for neurochemical sampling (50). The authors estimated with this method that atropine migrates about 10 times faster than sulpiride. Thus, it is conceivable that a rapid reduction in atropine concentration at the injection site increases the rate of atropine–muscarinic receptor dissociation leaving cholinergic receptors available for the ACh released by sulpiride, which may still be present in concentrations high enough as to produce this effect. It could be argued that the tardy hyperlocomotion developed after the combined treatment in the LH is partly due to atropine difusion toward the anterior hypothalamic/preoptic area where cholinergic mechanisms exerting an inhibitory action on locomotion have been demonstrated (1,2). However, this idea is unlikely, because of the relatively long distance for diffusion, and because administration of even a larger dose of atropine alone (18 μg) in the same LH region had no effect on locomotion (35). Finally, we do not have data to discard possible nonspecific effects induced by atropine that could be masking the early effects of sulpiride on locomotion, but the animals did not look with signs of maladise after atropine administration.

In summary, the present study supports the argument for a hypothalamic mechanism that controls locomotion, and suggests that such a mechanism is cholinergically driven, probably through muscarinic receptors, and connected with dopaminergic locomotor projections to the NAc. In addition, Lh sulpiride-induced locomotion probably acts via this system judging by the facts that LH sulpiride (a) released local ACh, and (b) caused locomotion that (c) was blocked by atropine. The main corollary of these results is that DA acts at D_2 receptors to exert an inhibitory control on cholinergic neurons or terminals in the LH, thereby inhibiting locomotion. Given the evidence that LH DA inhibits eating (26,27) and sulpiride causes eating and reinforces behavior (37), the LH locomotion system is probably related in part to procuring food and reinforcing eating behavior.

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